approach will be broadly applicable for studies of other macromolecular machines.

989-Pos Board B744

Dual Focus Fluorescence Cross-Correlation Spectroscopy for the Investigation of Biomolecule Folding and Binding in Flowing Liquids

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Fluorescence correlation spectroscopy measures the time constants for rate processes giving rise to fluorescence intensity fluctuations observed from one or more microscopic sub-volumes of a nanomolar aqueous solution containing the biomolecules of interest. In our approach, the analyte molecules flow through an electrophoresis capillary under the influence of pressure driven flow or electrophoretic flow and are probed by two spatially offset probe volumes in such a way that the molecules flow sequentially from one probe volume to the next. Fluorescence fluctuations are analyzed from each probe volume independently using autocorrelation analysis, and from the two spatially offset probe volumes using cross-correlation analysis. In this way, we are able to resolve fluorescence fluctuation time constants arising from diffusion, flow, triplet blinking, and conformational fluctuations. Conformational fluctuations are monitored by quenching and unquenching of dye-quencher molecules attached to DNA or RNA hairpin structures and probe the folding and unfolding kinetics of the hairpins. In this presentation, we will discuss recent results that show how base stacking within the loop region of the DNA and RNA hairpins alters the kinetics and thermodynamic stability of the hairpins. We will also discuss binding and unbinding of counterions to individual nucleotides as they flow through the capillary under the influence of an applied electric field. Emphasis will be placed on how the desired information can be extracted using our unique approach to fluorescence correlation spectroscopy.

990-Pos Board B745

A Single-Molecule Study of Toll-Like Receptor 4 Structure and Signalling

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In this study, we use a single-molecule fluorescence approach to image the reorganisation on the surface of live cells of individual fluorescently labelled Toll-like Receptor 4 (TLR4) molecules during signalling. TLR4, a key membrane protein in the innate immune system, is involved in the recognition of microbial pathogens, by detecting the presence of the lipopolysaccharide (LPS) component of exogenous Gram-negative bacteria.
Single-molecule tracking experiments will be described that allow us to follow changes in the diffusion of TLR4 and its oligomerisation state over a period of 30 minutes following addition of LPS. These studies provide new insights into how the TLR4 receptor is organised on the cell surface and cooperatively reorganises on binding LPS to trigger downstream signalling and modulate the immune response.

991-Pos Board B746

Maximizing the Fluorescence Signal and Photostability of Fluorophores by Quenching Dark-States

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Due to its easy detectability fluorescence is widely used in spectroscopy to investigate a variety of chemical and biochemical samples. The characteristics of fluorescence like intensity, lifetime, anisotropy and quantum yield contain information about electronic structure, mobility and orientation of fluorophors. The precision of fluorescence signal is limited by the number of detected fluorescence photons. Furthermore, experiments that require high time resolution for investigations of protein folding and dynamics are generally limited by the photon flux. Hence it is important to investigate and extend the fluorescence photon emission capabilities of fluorophors. In this study, three different additives which enhance the fluorescence signal were investigated as selective quenchers for triplet or radical states of Rhodamine 110 (Rh 110).
Fluorescence correlation spectroscopy (FCS) in combination with power plot analysis was used to describe the entire fluorescence output according to a derived kinetics model for excitation and fluorescence of Rh 110. The application of additives effectively prevented the triplet and radical formation of Rh 110 even at high excitation irradiance in the range of MW/cm^2 leading to more than ten times increased fluorescence countrate. Furthermore we demonstrate that additives also increase the fluorescence signal of labeled biomolecule.

992-Pos Board B747

Scanning Fluorescence Correlation Spectroscopy in Mitochondria of Living Cells

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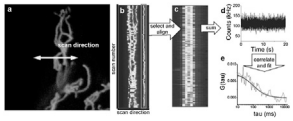
New methods to quantify dynamics and interactions of intracellular species provide key insights in cell biology. Fluorescence Correlation Spectroscopy (FCS) utilizes temporal autocorrelation of fluorescence fluctuations to study the dynamic properties of labeled molecules. Previous studies characterize diffusion and interaction of proteins in the nucleus and cytosol using FCS. However, only few deal with tubular organelles like the endoplasmic reticulum and mitochondria. The ability to accurately place the confocal volume in these dynamic organelles limits point FCS in vivo. Originally applied in membranes, scanning FCS (SFCS) addresses these challenges.
We applied SFCS to measure concentration and dynamic properties fluorescently-labeled molecules in mitochondria. SFCS accurately positions the confocal volume (in the x-y plane) by moving it along a linear scanning path. At a scanning orientation perpendicular to the mitochondria, we can also reduce photobleaching due to the brief residence times in the confocal volume.



General scheme of SFCS. (a) shows a fluorescence image of a mitochondria. The scan direction is oriented perpendicular to the mitochondria. (b) shows the acquired data before selection of mitochondria(orange box) and alignment(c). (d) represents the fluctuation trace, which is correlated and fitted to diffusion models (e).

993-Pos Board B748

Parallel Single-Molecule Excitation Spectroscopy of Gold Nanorods

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Gold nanorods show intense two photon luminescence arising from surface plasmon resonances (SPRs). Such SPRs exhibit a strong dependence on the environmental conditions and can be used to detect interactions between a nanorod and single biomolecules. Here we report a novel technique to acquire excitation spectra of multiple single gold nanorods in parallel, within a few seconds. We acquired two-photon excitation spectra of tens of single gold nanorods, and analyzed individual gold nanorod features. 3-dimensional fitting yields a spectral resolution of 1 nm. This allows for discriminating between single and multiple gold nanorods, as well as analysis of spectral changes in time. Using this technique we aim to detect single protein-gold nanorod interactions within a living cell.

994-Pos Board B749

Single-Cell Single-Molecule Co-IP Analysis

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Recent advances in single molecule imaging technique, such as single molecule Co-IP, have allowed us to probe interactions between weakly binding

proteins in cell lysates. In the single molecule Co-IP technique, bait proteins in the cell lysates were pulled down onto the substrate via surface immobilized antibodies. In this study, in stead of using cell lysates as a probe, single Co-IP technique is applied to single cell bait proteins. Our approach gives cell to cell variation information which was previously averaged out during lysis process. Activities of cells between different cell lines were also observed. This technique would provide detailed picture of protein interactions in cells.

995-Pos Board B750

Investigation of Diffusion in Structured Samples using Fluorescence Pair Cross Correlation

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Fluorescence Fluctuation Spectroscopy methods are powerful tools for studying diffusion both in vitro and in vivo. They are, however, limited to either homogeneous samples or to fit models that rely on generalized assumptions about the heterogeneity of the environment. Actual biological samples, however, often do not fulfill these conditions. The plasma membrane, for example, shows compartmentalization that changes dynamically [1]. This high level of complexity makes evaluation of results difficult.

To better understand the effects of heterogeneities such as structures or obstacles on molecular diffusion, we monitored the diffusion of lipids in a simplified model system. We used a lipid bilayer on a nanostructured surface [2] as a simplified system to study the effect of compartmentalization on diffusion. Our samples consisted of hexagonally arranged compartments of lipid bilayers that are connected via nanometer gaps in between the metal structures. By calculating the spatial pair cross correlation functions [3] of a signal acquired while rapidly scanning over the sample, we could measure the time of diffusion of fluorescently labeled lipids between partitions. Since both diameter of the compartments and size of the gaps can be adjusted, this system allows us to study the influence of these parameters on diffusion. Furthermore, we performed Monte Carlo simulations of diffusion in similar structures. This way, we could test the effects of different interactions of the sample and the environment on the correlation functions.

[1] T. Fujiwara et al., Journal of Cell Biology 2002, 157, 1071.
[2] T. Lohmueller et al., Nano Letters 2012, 12, 1717.
[3] M. Digman et al., Biophysical Journal 2009, 97, 665.

996-Pos Board B751

Use of Cyclic Olefin Polymer in Single Molecule Total Internal Reflection Fluorescence Microscopy

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Single molecule imaging is an established technique for many researchers in the biophysical sciences. Often, a fluorescent molecule bound near a glass or quartz surface is excited using total internal reflection fluorescence (TIRF) microscopy. TIRF excitation selectively excites fluorophores at the surface of a well or channel, thereby eliminating background fluorescence from bulk solution. However, nonspecific binding of fluorescent probes and fluorescently labeled molecules can become a limiting factor for protein association experiments. Extensive cleaning is required when using a glass or quartz substrate and the substrate is often coated with polymers such as poly-ethylene-glycol (PEG) to reduce non-specific binding. To eliminate the extensive wash and coating procedure steps we use a plastic cyclic olefin copolymer (COC) substrate that is extremely hydrophobic and can be passively activated to perform specific protein-protein interaction experiments in a very simple manner. Furthermore, COC has optical properties similar to glass making it amenable to TIRF microscopy. We first show simple processes to activate the surface in under a minute while rejecting fluorescent labeled antibody at concentrations of 1 nM. This is confirmed using objective-based TIRF microscopy and off-the-shelf COC microfluidic channels. We also show sufficient signal to noise to observe green fluorescent protein at the single molecule level using TIRF excitation. Finally, activated surfaces are used to perform a protein association experiment by detecting a sandwich of two antibodies bound to a single antigen molecule. We believe fluidic devices made of COC polymer will eliminate many of the preparation steps employed to treat other substrates allowing for easier and significantly faster protein association experiments.

997-Pos Board B752

Simultaneous Position and Orientation Imaging of Polarized Fluorescence from Rod-In-Rod Semiconductor Nanoparticles on Cytoplasmic Dynein

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Sub-pixel particle tracking and polarized total internal reflection fluorescence (polTIRF) microscopy have been instrumental in understanding of motor protein function. We have developed a method combining sub-pixel tracking and polTIRF microscopy to simultaneously track position and orientation of single fluorescent particles. The sample is illuminated with circularly polarized light, and the emission is split into four polarizations and imaged with an EMCCD camera. The Yanagida lab recently published such a technique (Ohmachi et al, PNAS vol. 109, 2012). Our lab has developed a similar method, incorporating calibration routines to compensate for depolarization by phase shifts at reflecting surfaces and wavelength- and polarization-dependent detection efficiencies of the camera channels. The application of a 4x4 calibration matrix, generated by measuring deviations from theoretical polarized fluorescence intensities, compensates for depolarization caused by reflecting surfaces and channel crosstalk. Normalizing to an unpolarized sample at the relevant emission wavelength accounts for wavelength-dependent differential sensitivity of the four detection channels. Rod-in-rod CdSe core, CdS shell particles exhibit high polarization ratios; rods with dimensions of only ~5x20 nm display an average polarization ratio of 0.81. This position and orientation tracking method is currently being used to observe rotations of streptavidin-conjugated quantum rods bound to the cytoplasmic dynein AAA ring. Correlation of angular and translocation information may provide insight into mechanisms of fluctuating dynein stepping. Supported by NIH grant P01GM087253.

998-Pos Board B753

Revealing Myosin's Power Stroke with High-Speed Scattering Interferometry

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How nature addresses brownian motion and achieves high processivity of molecular motors is a fundamental question in biophysics. Single molecule techniques are particularly suited to investigate nanoscopic motion with high spatial accuracy, but have struggled to provide information about structural dynamics associated with the individual motor steps. Fundamentally, the challenge has been to achieve both nanometer spatial accuracy and microsecond temporal resolution without considerably interfering with the mechanics of the step. Here, we show that interferometric scattering microscopy (iSCAT) can track the motion of both the head and stalk of myosin 5a in vitro without need of a drastic reduction of ATP concentration. We achieved 1 nm spatial precision and sub-millisecond temporal resolution using quantum dot-sized metallic nano-particles as labels. In this way, we could visualize conformational changes during the myosin 5 processive movement, a waiting state and brownian search when encountering obstacles - fundamental components of the stepping mechanism of myosin 5a. Remarkably, we observe tight control of lever-arm diffusion and motion for the duration of the step. Moreover, iSCAT signal brings additional information about z-position of the label which completes the 3D picture of myosin's moment. Our results illustrate how iSCAT can be used as a new approach to study dynamic structural changes of biomolecules in action.

999-Pos Board B754

Nanoparticle Probes of Cell Surface Molecule Rotation

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Rotation of membrane proteins is a sensitive measure of their aggregation state and their interactions with other membrane species. We have used